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DETECTION OF MUTAGENIC ACTIVITY:

SCREENING OF TWENTY-THREE ORGANIC COMPOUNDS OF INDUSTRIAL ORIGIN IN THE ST. CLAIR RIVER

NOVEMBER 1979

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The Honourable
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Minister

Graham W. S. Scott, Q.C.,
Deputy Minister

Date Due

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DETECTION OF MUTAGENIC ACTIVITY:

SCREENING OF TWENTY-THREE ORGANIC COMPOUNDS OF INDUSTRIAL ORIGIN IN THE ST. CLAIR RIVER

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NOV 20 1979

EXPLANATORY NOTE

"THE DETECTION OF MUTAGENIC ACTIVITY; SCREENING OF TWENTY-THREE COMPOUNDS OF INDUSTRIAL ORIGIN"

One of the objectives of the St. Clair River Organics Study is to determine, using bacterial tests, the mutagenic properties of organic compounds contained in various industrial and municipal effluents discharged to the St. Clair River.

Environment Ontario scientists used the Ames Test to achieve this testing capability. The Ames Test, developed by American scientist, Bruce Ames, is a bacterial test which detects mutagenic compounds. Mutagenic compounds alter genetic information and result in a permanent heritable genetic change. It has been shown through other research that some compounds that are mutagenic may also have carcinogenic properties.

The report entitled "The Detection of Mutagenic Activity; Screening of Twenty-three Compounds of Industrial Origin" describes the results of laboratory testing of 23 of approximately 50 industrial compounds found in the St. Clair River. In the Ministry's Toronto laboratory, chemically pure forms of these compounds were assessed at concentrations considerably higher than those found in the industrial effluents. On this basis five of these compounds were found to be mutagenic: dichloromethane; 1,1 - dichloroethane; 1,2 - dichloroethane; 1,2 - bromochloroethane; 1,2,3 - trichloropropane. The remainder of these 50 industrial compounds are currently under analysis.

The report indicates that some compounds present in waste discharges to the St. Clair River System are mutagenic in their pure chemical form. The next phase of this study, laboratory testing of actual municipal and industrial effluents for their mutagenic activity, is now underway and will be completed this year. This work will assess the levels, sources and extent of possible mutagenic activity throughout the St. Clair River system which will be essential in evaluating the need for additional abatement measures.

...2

A search of scientific literature with respect to the possible health effects of the 50 compounds will be undertaken. Depending on these findings additional research into the health effects may be undertaken as part of the St. Clair River study.

"The Detection of Mutagenic Activity; Screening of Twenty-three Compounds of Industrial Origin" is one of six publications being published by the Ontario Ministry of the Environment's St. Clair River Organics Study Group. This group is composed of Environment Ontario scientists and analysts, who have worked on the waterway since 1977. Other reports published by the group include "Biological Surveys 1968 and 1977" and "Waste Dispersion".

The three remaining studies to be completed and published in early 1980 by the study group deal with the identification and quantification of industrial organic compounds, fish toxicity and tainting assessment of industrial effluents, and biodegradation of organic compounds in the St. Clair River system.

FOREWORD

Investigation of organic chemicals in industrial effluents along the St. Clair River commenced in 1975 after the adoption of gas chromatography - mass spectrometry analytical methodologies by the Laboratory Services Branch of the Ministry of the Environment made the detection of low concentrations of such compounds possible. In a preliminary report, 'Organic Compounds of Industrial Origin in the St. Clair River', developed as an internal working document in January of 1977, positive identification was made of some 47 organic compounds discharged to the St. Clair River.

The St. Clair River Organics Study Group was formed early in 1977 in response to concerns about the presence and significance of the organic compounds identified, particularly in relation to possible effects on public water supplies downstream and the potential bio-accummulation of such compounds by fish resident in or frequenting this important waterway. Also, it was felt that a comprehensive assessment of water quality conditions and aquatic life was warranted to permit a comparison with unpublished biological data collected in 1968.

Representation on the Study Group included staff from various branches of the Ministry of the Environment and the Special Studies and Services Branch of the Ministry of Labour. Early in its deliberations, the Study Group identified the following goal:

> *To assess the presence and significance of organic chemical compounds in the St. Clair River system and to establish from this programme recommendations for control measures and further studies that may be required in relation to human health and environmental effects.*

In order to accomplish the above goal, the following ten objectives were identified:

1. Identification and quantitation of organic compounds in industrial discharges, St. Clair River water, potable water supplies, bottom sediments and fish.

2. Completion of a literature search on the characteristics and effects of the organic compounds identified. Lethal, sub-lethal and synergistic effects of these compounds on human and aquatic life to be considered.

3. Determination of the significance of chlorinating trace amounts of these organic materials in industrial cooling water discharges, sewage plant effluents and in potable water supplies.

4. Determination of the types and concentrations of compounds causing fish toxicity and fish tainting problems.

5. Estimation of the dissolved organics concentration at any point in the river based on effluent quality and flow measurements.

6. Completion of benthic macroinvertebrate, aquatic plant, sediment and water quality studies on the river in terms of biological and common chemical indicators. Results to be compared to survey work carried out in 1968-69. Assessments of mercury levels in sediments to be completed.

7. Determination of the potential carcinogenicity of organic compounds through quick-screening microbiological tests for mutagenesis.

8. Establishment of whether biodegradation or alteration of organic compounds occurs through bacterial action in sediments.

9. Development of recommendations clarifying levels to which contaminants must be reduced, as a basis for necessary treatment and effluent controls.

10. Clarification of the need for additional research to establish potential dangers to human health or the natural environment where this information is lacking.

Work on the component studies related to these objectives progressed throughout 1977 and 1978 and into 1979. As a result of these efforts the following reports will be published during 1979, to form an integrated series covering the objectives defined previously.

(1) Identification and Quantitation of Organic Compounds.
(2) Fish Toxicity and Tainting Evaluations for Selected Industrial Effluents.
(3) Waste Dispersion.
(4) Biological Surveys 1968 and 1977.
(5) The Detection of Mutagenic Activity; Screening of Twenty-three Compounds of Industrial Origin.
(6) Biodegradation of Organic Compounds.

Wherever possible, each of these reports will present conclusions and advance recommendations to achieve an improvement of water quality in the St. Clair River system or to clarify additional research requirements in regard to potential human health or environmental effects.

It is anticipated that subsequent reports dealing with fish toxicity and tainting and the potential carcinogenicity of industrial organic compounds in relation to the St. Clair River will be issued by the Ministry, since these particular components will be the subject of further study by the Limnology and Toxicity Section of the Water Resources Branch and the Microbiology Section of the Laboratory Services Branch, respectively.

Table of Contents

Page

Summary 1

Introduction 2

Theory of Mutagenicity Detection 3

Screening of Compounds for Mutagenic Activity 6

Analytical Methods 6

Results and Discussion 7

Conclusion 16

References 29

Summary

1) A bacterial mutagenicity detection system, modelled after the "Ames" test, has been used to screen twenty-three pure organic compounds identified in industrial effluents entering the St. Clair River at Sarnia, Ontario. A known mutagenic compound, styrene oxide, not found in these effluents, was also analyzed as a positive control.

2) Mutagenic activity was detected in styrene oxide and in four of the twenty-three compounds. Presumptive mutagenic activity was identified in one additional compound. Corroborative evidence obtained from the literature supported the classification of two of four mutagenic compounds and the one presumptive mutagenic compound.

3) Mutagenic activity has been reported in the literature for an additional three of these compounds which had been scored non-mutagenic in our screening system.

4) Carcinogenic activity has been reported in the literature for seven of the twenty-three organic compounds entering the St. Clair River.

...2

Introduction

Mutagenic and/or carcinogenic compounds may enter the environment through several routes: (a) as a result of direct use in manufacturing, (b) as by-products of industrial processes, (c) as components of waste discharge streams in industrial and manufacturing processes. The consequences of exposure to carcinogenic compounds has been demonstrated in studies involving benzo(a)pyrene and vinyl chloride, by an elevated cancer incidence in man (1). Identification of carcinogens by their effect on man may, in effect, be too slow since manifestation of a cancer symptom may occur 5 to 30 years after exposure (1, 2). In our present state of relative ignorance in accurately predicting the impact on humans exposed to suspect chemicals, new avenues must be explored to quickly identify these hazardous compounds and to control or eliminate as many as possible from man's surroundings.

An important contribution to the detection of carcinogens was the demonstration of mutagenic activity in many carcinogenic substances (3). A detection system using bacteria as indicators was able to identify 64 to 85 percent (4, 5) of carcinogenic compounds as mutagenic while 98 percent of non-carcinogenic compounds manifested no mutagenic activity (5). This system is an inexpensive and rapid means of screening compounds and has been recommended (6, 7) as one of a battery of tests for the identification of mutagenic and potential carcinogenic properties of compounds..

In 1978 the Microbiology Section of the Laboratory Services Branch, Ministry of the Environment, began to develop the analytical capability to detect mutagenic substances. The first phase of this work was to bring on-line presently available techniques (4) for use in the screening of pure compounds. A detection system for mutagenic activity has now been developed and is described in a previous report (8).

...3

This detection system is presently being used to screen compounds of industrial origin in the aquatic environment of Ontario, and specifically the man-made organic compounds identified in industrial effluents entering the St. Clair River at Sarnia. Since the concentrations of specific organic compounds measured in these effluents were generally low (9), and since mutagenic activity of organic compounds is normally detected at concentrations in excess of 1 ug (5, 10), our initial tests have been conducted on pure compounds identified in the samples rather than on the samples themselves. This report describes the results of mutagenicity screening on twenty-four pure compounds, twenty-three of which have been identified in industrial effluents entering the St. Clair River.

Theory of Mutagenicity Detection

Mutagenic activity is detected using as indicators one of five genetically engineered strains of the bacterium Salmonella typhimurium (4). These strains have been modified to increase their sensitivity to mutagenic compounds (4, 8). They are unable to grow unless their medium is supplemented with histidine. If these bacteria are exposed to a mutagenic compound, some cells will be mutated restoring their ability to synthesize histidine (histidine revertants). These revertants are counted as colonies on an agar medium devoid of histidine. The strains will theoretically respond to a compound of higher mutagenic potency or to a larger dose (concentration) of a mutagenic compound by an increase in the number of histidine revertants.

Many mutagenic compounds require metabolic activation before they can be detected. The conversion of the compound to its mutagenically active metabolite is accomplished by the addition of mammaliam microsomal enzymes (S-9) to the detection system. These enzymes are usually obtained from rat liver induced with Aroclor 1254 or phenobarbitol.

...4

A finite number of histidine revertants are present in all indicator strains and these background revertants are routinely measured on negative control plates. Mutagenic activity is suspected when the number of revertants, produced in the indicator strains exposed to a compound, exceeds the number of background revertants by a critical amount. A quantitation of the mutagenic response is made using the mutagenic activity ratio (MUTAR) described by Commoner (5) and calculated by the formula:

$$\text{MUTAR} = (E\text{-}C)/C_{av}$$

Where E is the number of revertants produced in response to the compounds; C is the number of background revertants in the respective indicator strain; (E-C) is revertants in excess of background and C_{av} is the average number of background revertants calculated from the historical record of negative control plates.

The probability of correctly classifying a compound as mutagenic or non-mutagenic based on MUTAR values is shown in Table 1. If a 0.7 value was selected as a cut-off, many compounds would produce a response exceeding this value and the group would have a high probability ($p = 0.95$) of containing a mutagen. However, the probability of correctly identifying non-mutagenic compounds would be low ($p = 0.20$). At a cut-off value of 1.5, there is an equal probability ($p = 0.82$) of correctly classifying compounds of higher value as mutagenic and compounds of lower value as non-mutagenic. In this report a cut-off value of 2.5 was selected with a high probability ($p = 0.95$) of correctly classifying non-mutagenic compounds and $p = 0.81$ of correctly classifying a mutagen.

Table I. Probability Of Correctly Classifying a Presumptive Carcinogen (Mutagen) And Non-carcinogen (Non-mutagen) At Various Mutagenic Activity Ratios.

	Probability		
MUTAR $((E-C)/C_{av})$	0.7	1.5	2.5
Case I. Probability of correctly classifying a presumptive carcinogen (mutagen)	0.95	0.82	0.81
Case II. Probability of correctly classifying a non-carcinogen (non-mutagen)	0.20	0.82	0.95

* Calculated from optimal indicator strain, optimal concentration of test substance and metabolic (S-9) activation when necessary.

From Commoner (5)

...6

Screening of Compounds for Mutagenic Activity

Compounds were screened using the mutagenic spot test with four indicator strains in the presence and absence of metabolic (S-9) activation. Those compounds demonstrating signs of mutagenic activity (MUTAR values exceeding 1.5 or a ring of revertants surrounding the applied compound) were considered presumptive mutagens.

The mutagenic activity of a presumptive compound was confirmed using the composite mutagenic test, the most responsive indicator strain in the presence and absence of S-9 and with a minimum of 3 doses (concentrations) of the compound. Mutagenic activity was confirmed if a mutagenic dose-response relationship was observed.

Analytical Methods

The source of the bacterial indicator strains as well as their storage, culturing and quality control assessment are described in a previous report (8). Vogel-Bonner agar medium, top agar, S-9 microsomal enzymes and S-9 reaction mixture were prepared as suggested by Ames (4) and as described by Rokosh (8).

In the mutagenic spot test, a Vogel-Bonner agar plate was overlaid with top agar containing one of the indicator strains or an indicator strain plus S-9 reaction mixture. The test compound was applied directly on the surface of the overlay. Negative and positive control plates (8) were run in parallel with each tested compound. All plates were incubated in sealed containers at 37°C for 48 hours and colonies developing on all plates were counted.

The spot test was modified for compounds of low water solubility and boiling points exceeding 65°C. Such compounds were pre-incubated at room temperature for approximately 60 minutes in S-9 reaction mixture or in reaction mixture buffer minus enzymes (8). Aliquots of these pre-incubated compounds

...7

were then spotted on the overlaid plates. This modification improved contact with the compound and the S-9 enzymes and also the dispersion of the compound over the plate.

The composite mutagenic test (Ames pour plate) was used to confirm the presumptive mutagenic activity of a compound. In the composite test, the compound and indicator strain were mixed in top agar and overlaid on Vogel-Bonner plates. If metabolic activation was required, the S-9 reaction mixture was added to the top agar immediately before pouring. Procedures for the preparation of control plates, incubation and the counting of colonies were similar to those of the spot test with the exception that plates containing the same concentration of the test compound were isolated in separate sealed containers.

Results and Discussion

Samples of industrial effluents entering the St. Clair River at Sarnia, Ontario have been shown to contain man-made organic compounds (9) some of which (organohalides and aromatic hydrocarbons) are excellent candidates to be screened for mutagenic activity. In general, the concentrations of synthetic organics in these samples were low (9) and below the level of 1 to 100 ug/ml which were the dosages studied in bacterial mutagenicity (Ames) tests (5). We have assumed that the measured levels of these compounds would, without a concentration step, be below the detection limits of our screening system. Initially we have attempted to screen pure compounds identified in these effluents rather than screen the effluent samples.

Of the twenty-four compounds tested, twenty-three have been identified in industrial effluents entering the St. Clair River. Styrene oxide, a known mutagen (10) was not identified in these effluents but was also screened and was considered as a positive control. The two isomers of dichloroethane, 1,1- and 1,2-dichloroethane and the two isomers of propanol, 1- and 2-propanol were

...8

screened as independent compounds. Dichloroethane and propanol have been found in these effluents (9) but their isomeric forms were not identified.

The list of those compounds tested, their purity and their toxicity to the indicator strains are presented in Table 2. These compounds as well as information on their purity were provided by the Organics Trace Contaminents Section. The toxic limits were experimentally determined using the degree of bacterial lawn inhibition in the mutagenic spot test. The maximum dose of each compound tested was below the toxic dose of that compound.

The results of the screen by the mutagenic spot test of these twenty-three compounds plus styrene oxide are shown in Table 3. The compounds dichloromethane, 1,1-dichloroethane, 1,2-dichloroethane, 1,2-bromochloroethane, 1,2,3-trichloropropane and styrene oxide produced elevated revertants in stain TA 1535 or TA 100. TA 1535 and TA 100 are strains sensitive to compounds such as methyl-methanesulphonate (11) which is a mutagenic alkylating agent (12). Excess revertants occurred in strain TA 98 when exposed to dichloromethane, 1,1-dichloroethane and 1-propanol. However, its parent strain TA 1538 did not respond to any of these three compounds. TA 1538 and TA 98 are sensitive to compounds such as 2-nitrofluorene (11). The response of strain TA 98 to the above three compounds was considered an anomaly of strain TA 98 (8) and further testing will be required.

Metabolic (S-9) activation did not increase the potency of 1,1-dichloroethane, 1,2-dichloroethane and 1,2-bromochloroethane, but 1,2,3-trichloropropane, dichloromethane and styrene oxide produced more revertants in strain TA 1535 when activated by S-9. The failure of styrene to produce elevated revertants in TA 1535 even with S-9 activation would suggest styrene oxide was not formed by Arochlor induced microsomal enzymes.

...9

Table 2. Purity And Toxicity * Of Test Compounds

Compound	Toxic concentration of compound µ moles (µ litres) / plate	Purity %	Contaminant
Dichloromethane	> 393 (> 25)	99	
Chloroform	312 (25)	98-99	Ethanol
Carbon tetrachloride	260 (25)	99	
Bromoform	11 (1)	99	
1,1-Dichloroethane	> 298 (> 25)	99	
1,2-Dichloroethane	> 318 (> 25)	99	
1,2-Bromochloroethane	> 61 (> 5)	99	
1,1,1-Trichloroethane	253 (25)	99	
1,1,2,2-Tetrachloroethane	48 (5)	99	
1,1,2-Trichloroethene	> 278 (> 25)	99	
Tetrachloroethene	245 (25)	99	
1,2,3-Trichloropropane	46 (5)	99	
Ethanol	> 428 (> 25)	95	Water
1-Propanol	> 325 (> 25)	99	
2-Propanol	> 65 (> 5)	99	
_t_-Butanol	> 265 (>25)	99	
2-Butanone	> 280 (> 25)	99	
Benzene	> 281 (> 25)	99	
Toluene	> 47 (> 5)	99	
Styrene	> 337 (> 25)	98	_t_-butylpyrocatechol
Styrene oxide	88 (10)	99	
Cumene	180 (25)	99	
_o_-Dichlorobenzene	222 (25)	99	
_m_-Dichlorobenzene	219 (25)	99	

* * Toxic concentration was arbitrarily chosen as that quantity of compound resulting in a clearing of the bacterial lawn over at least 25% of the surface area of a VB plate.

...10

Table 3. Mutagenic Response Of The Spot Test To Test Compounds

				Revertants (in excess of background) per plate			
Compound	Trials	Conc. per Plate (μ moles)	S-9	TA 1535	TA 1538	TA 98	TA 100
Dichloromethane	2	393	-	7	1	40	119
	1	393	+	13	0	-	294
Chloroform	2	62.5	-	2.5	1	2	0
		62.5	+	9	0	-	0
Carbon tetrachloride	2	52	-	6.5	0	7	5.5
	1	52	+	4	0	-	0
Bromoform	1	5.7	-	8	0		0
	1		+	15	0		0
1,1-Dichloroethane	2	298	-	26	1.5	11	138
	1	298	+	9	0	-	77
1,2-Dichloroethane	3	318	-	171	0	3	182
	1	318	+	113	0	-	256
1,2-Bromochloroethane	1	30	-	309	0	-	561
	1	30	+	307	27	-	569
1,1,1-Trichloroethane	1	101	-	5	2	0	24
	1	101	+	11		0	2
1,1,2,2-Tetrachloroethane	1	9.5	-	8	-	-	0
	1	9.5	+	0	-		0
1,1,2-Trichloroethene	1	111	-	4	-	5	0
	1	111	+	0	-	-	0
Tetrachloroethene	1	98	-	8	1	7	0
1,2,3-Trichloropropane	1	4.6	-	27	0	-	-
	1	4.6	+	130	19	-	-
Ethanol	1	86	-	2	1	0	-
	1	86	+	7	0	0	-
1-Propanol	3	325	-	0	0	12	20
	1	325	+	1	-	0	19
2-Propanol	1	65.4	-	6	4	0	0
	1	65.4	+	0	1	0	0

...11

Table 3. continued.

				Revertants (in excess of background) per plate			
Compound	Trials	Conc. per Plate (μ moles)	S-9	TA 1535	TA 1538	TA 98	TA 100
t-Butanol	1	265	-	2	0	0	-
	1	265	+	0	4	4	-
2-Butanone	1	280	-	0	0	0	-
	1	280	+	3	0	4	-
Benzene	1	281	-	0	3	0	-
	1	281	+	2	11	4	-
Toluene	1	47	-	8	0	0	0
	1	47	+	0	6	0	0
Styrene	3	67.4	-	5	1	0	2
	2	67.4	+	3	7	0	1
Styrene oxide	1	8.8	-	367	-	-	1184
	1	8.8	+	576	-	-	1595
Cumene	2	36.0	-	10	5	2.5	22
	1	36.0	+	9	3	0	0
o-Dichlorobenzene	1	44.4	-	0	0	1	0
	1	44.4	+	0	-	0	0
m-Dichlorobenzene	2	43.8	-	0	0	1.5	0
	1	43.8	+	-	0	0	0
Background revertants			-	14	5	4	121
			+	14	9	16	168

...12

The mutagenic dose-response relationship of twenty of the twenty-four screened compounds have been measured and the results are shown in Figures 1 - 20. The mutagenic response has been measured using the composite mutagenic test but where possible the results of the mutagenic spot test have also been included. MUTAR values have been calculated for all twenty-four compounds from the optimal indicator strain, optimal concentration of compound tested, S-9 activation if necessary and from either the spot or composite mutagenic test. These MUTAR values are shown in Table 4.

A mutagenic dose-response relationship was observed with 1,1-dichloroethane (Figure 2), 1,2-dichloroethane (Figure 3), 1,2-bromochloroethane (Figure 4), 1,2,3-trichloropropane (Figure 5) and styrene oxide (Figure 18). Metabolic (S-9) activation of 1,2,3-trichloropropane and styrene oxide increased their mutagenic response. The MUTAR values of the above five compounds exceeded the critical values of 2.5 (Table 4). Based on a dose-response relationship and MUTAR values exceeding 2.5, the above five compounds were classified mutagenic.

Dichloromethane (Figure 1) produced a dose-response relationship with strain TA 1535. MUTAR values of this compound exceeded 1.5 but were less than 2.5 (Table 4). Problems were encountered with dichloromethane because its boiling point (40^{o}C) was less than the top agar temperature in the composite test (42-45^{o}C) and the majority of the compound was probably lost in the initial stages of the test. However, based on a positive dose-response relationship but sub-critical MUTAR values, dichloromethane was considered a presumptive mutagen.

A dose-response relationship was also observed with S-9 activated 1,1,1-trichloroethane (Figure 10) and tetrachloroethene (Figure 13). MUTAR values of both S-9 activated compounds were below 1.5 (Table 4). A mutagenic response of twenty-three revertants per plate was measured with 5 µl unactivated tetrachloroethene (Figure 13) resulting in a MUTAR value of 1.64, however, this

...13

Table 4. Mutagenic Activity Ratio (MUTAR) Values of Test Compounds

Compound	Strain with Highest MUTAR Value	S-9	MUTAR Value
Dichloromethane	1535	-	2.14
Chloroform	1535	-	0.71
Carbon tetrachloride	1535	-	0.50
Bromoform	1535	+	1.14
1,1-Dichloroethane	1535	-	2.93
1,2-Dichloroethane	1535	-	16.50
1,2-Bromochloroethane	1535	-	35.57
1,1,1-Trichloroethane	1535	+	0.86
1,1,2,2-Tetrachloroethane	1535	-	0.57
1,1,2-Trichloroethene	1535	-	0.50
Tetrachloroethene	1535	+	1.14
1,2,3-Trichloropropane	1535	+	9.29
Ethanol	1535	+	0.50
1-Propanol	100	-	0.17
2-Propanol	1538	-	0.80
t-Butanol	1538	-	0.40
2-Butanone	1538	-	0.60
Benzene	1538	+	1.22
Toluene	1538	+	0.66
Styrene	1535	-	0.93
Styrene oxide	1535	+	41.14
Cumene	1535	-	0.71
o-Dichlorobenzene	1535	-	1.07
m-Dichlorobenzene	1535	-	0.79

...14

response greatly exceeded values at all other unactivated concentrations tested and was assumed to be in error. Based on MUTAR values less than 1.5, both the above compounds were classified non-mutagenic.

The compounds chloroform (Figure 6), carbon tetrachloride (Figure 7), bromoform (Figure 8), 1,1,2,2-tetrachloroethane (Figure 9), 1,1,2-trichloroethene (Figure 11), ethanol (Figure 12), t-butanol (Figure 14), 2-butanone (Figure 15), benzene (Figure 16), styrene (Figure 17), o-dichlorobenzene (Figure 19) and m-dichlorobenzene (Figure 20) demonstrated no dose-response relationships. MUTAR values of all the above compounds fell below 1.5 (Table 4) and the above compounds were classified non-mutagenic. MUTAR values of 1-propanol, 2-propanol, toluene and cumene (Table 4) also fell below 1.5 and were also considered non-mutagenic.

When the results of our screening system was compared with the mutagenic analyses of these compounds as reported in the literature (Table 5), mutagenic activity was correctly identified in 1,2-dichloroethane, 1,2-bromochloroethane and styrene oxide. Dichloromethane, identified as a presumptive mutagen in our screen, has been reported as mutagenic. The compounds chloroform, carbon tetrachloride, ethanol and benzene were correctly identified non-mutagenic. The reported mutagenic activity of 1,1,2,2-tetrachloroethane and the weakly mutagenic 1,1,2-trichloroethene have not been detected in our screening system. However, the two former compounds were toxic at doses of 5 µl per plate. Simmon and Tardiff (14) suggested that such compounds be tested using a reduced exposure time and these compounds will be re-examined. The observed mutagenic activity of 1,1-dichloroethane and 1,2,3-trichloropropane could not be substantiated in the literature.

...15

Table 5. Comparison of Mutagenic Classification of Compounds Screened by the Biohazards Unit and Mutagenic and Carcinogenic Activity Reported in the Literature.

Compound	Biohazards Mutagenic Activity Scoring	Reported		Reference
		Mutagenic Activity	Carcinogenic Activity	
Dichloromethane	Pres. +	+	(+) *	(13)
Chloroform	-	-	+	(13)
Carbon tetrachloride	-	-	+	(13, 10)
Bromoform	-	+	+	(13)
1,1-Dichloroethane	+			
1,2,-Dichloroethane	+	+	+	(13, 14)
1,2-Bromochloroethane	+	+		(14)
1,1,1-Trichloroethane	-			
1,1,2,2-Tetrachloroethane	-	+		(14)
1,1,2-Trichloroethene	-	(+) #	+	(13)
Tetrachloroethene	-			
1,2,3-Trichloropropane	+			
Ethanol	-	-	-	(10)
1-Propanol	-			
2-Propanol	-			
t-Butanol	-			
2-Butanone	-			
Benzene	-	-	+	(15)
Toluene	-		A	(15)
Styrene	-			
Styrene oxide	+	+	-T	(10)
Cumene	-			
o-Dichlorobenzene	-			
m-Dichlorobenzene	-			

+ Positive, - Negative, * Carcinogenic activity detected only in tissue culture assay,

# Mutagenic activity less than 2.5,

A Enhances carcinogenic response of 7,12-dimethylbenze(a)antracene,

-T Under test.

Conclusion

Table 5 demonstrates the correlation between carcinogenic and mutagenic activity of dichloromethane, bromoform, 1,2-dichloroethane and 1,1,2-trichloroethene. Dichloromethane and 1,2-dichloroethane have been identified as mutagenic in our screening systems. Table 5 also contains 3 carcinogenic compounds which are not mutagenic, chloroform, carbon tetrachloride and benzene, which could not be detected in the bacterial mutagenicity test. The mutagenic compounds bromoform, 1,1,2,2-tetrachloroethane and 1,1,2-trichloroethene could not be detected in our mutagenicity screen but these may be detected by refinements in our analytical systems. The presence of these undetected mutagenic compounds underlines the need for a battery of mutagenicity tests to be used in the screening of compounds or samples. Additional tests are presently under investigation and will be implemented as soon as possible.

The results of the mutagenic screen of twenty-three man-made compounds identified in industrial effluents entering the St. Clair River are in this interim report; and the remaining compounds (9) are being analyzed. The high incidence of mutagenic compounds among the twenty-three tested does not forecast a high incidence of mutagenic compounds in the remainder to be screened, but rather reflects a-priori selection of compounds expected to be mutagenic. The final interpretation of the number of mutagenic compounds among those identified in these effluents awaits the completion of the screening of all compounds. The final conclusions will be reported at a later date.

...17

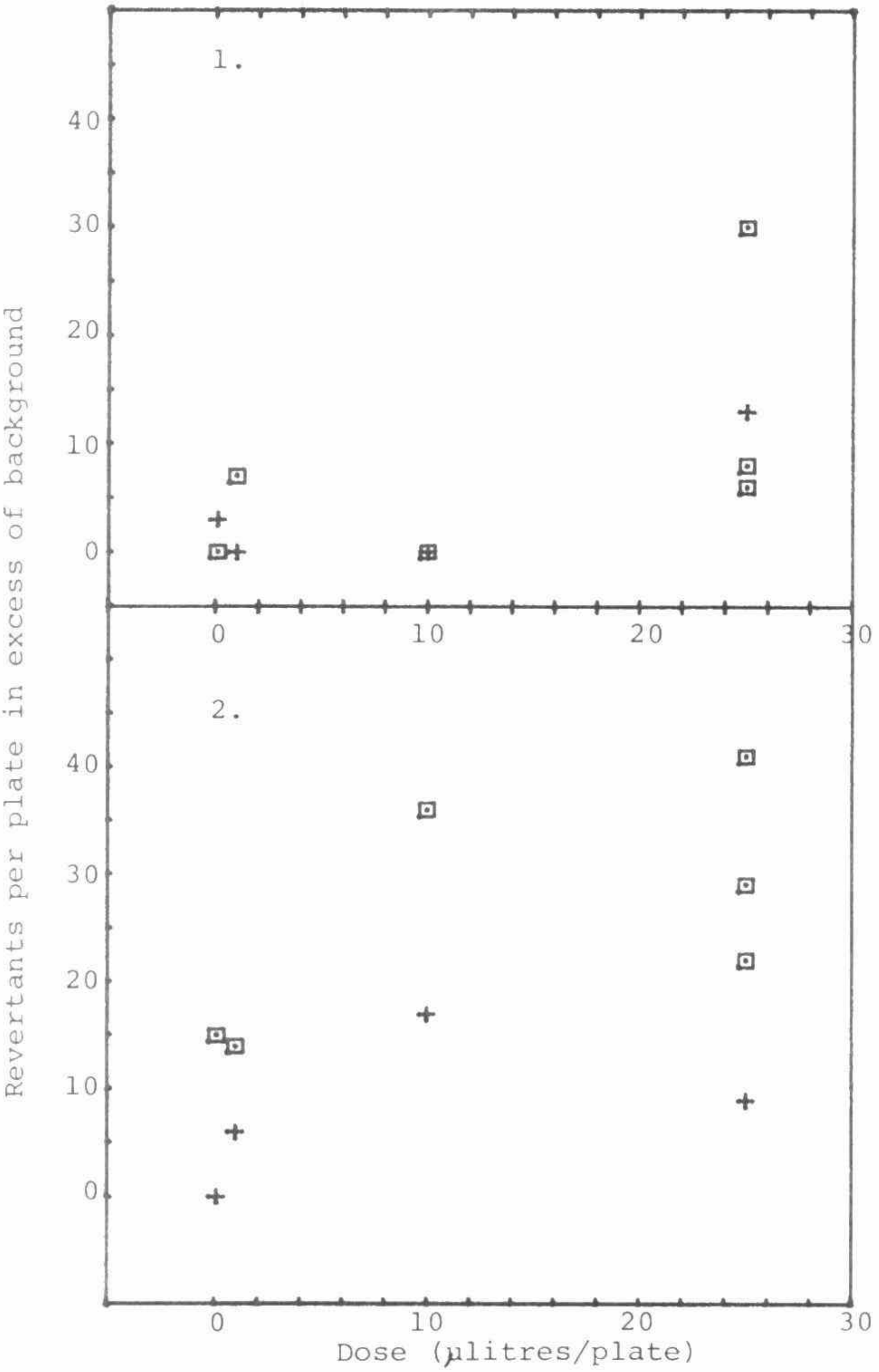

Figure 1. Mutagenic dose-response relationship of dichloromethane with TA 1535

Figure 2. Mutagenic dose-response relationship of 1,1-dichloroethane with TA 1535

▣ unactivated, + S-9 activated

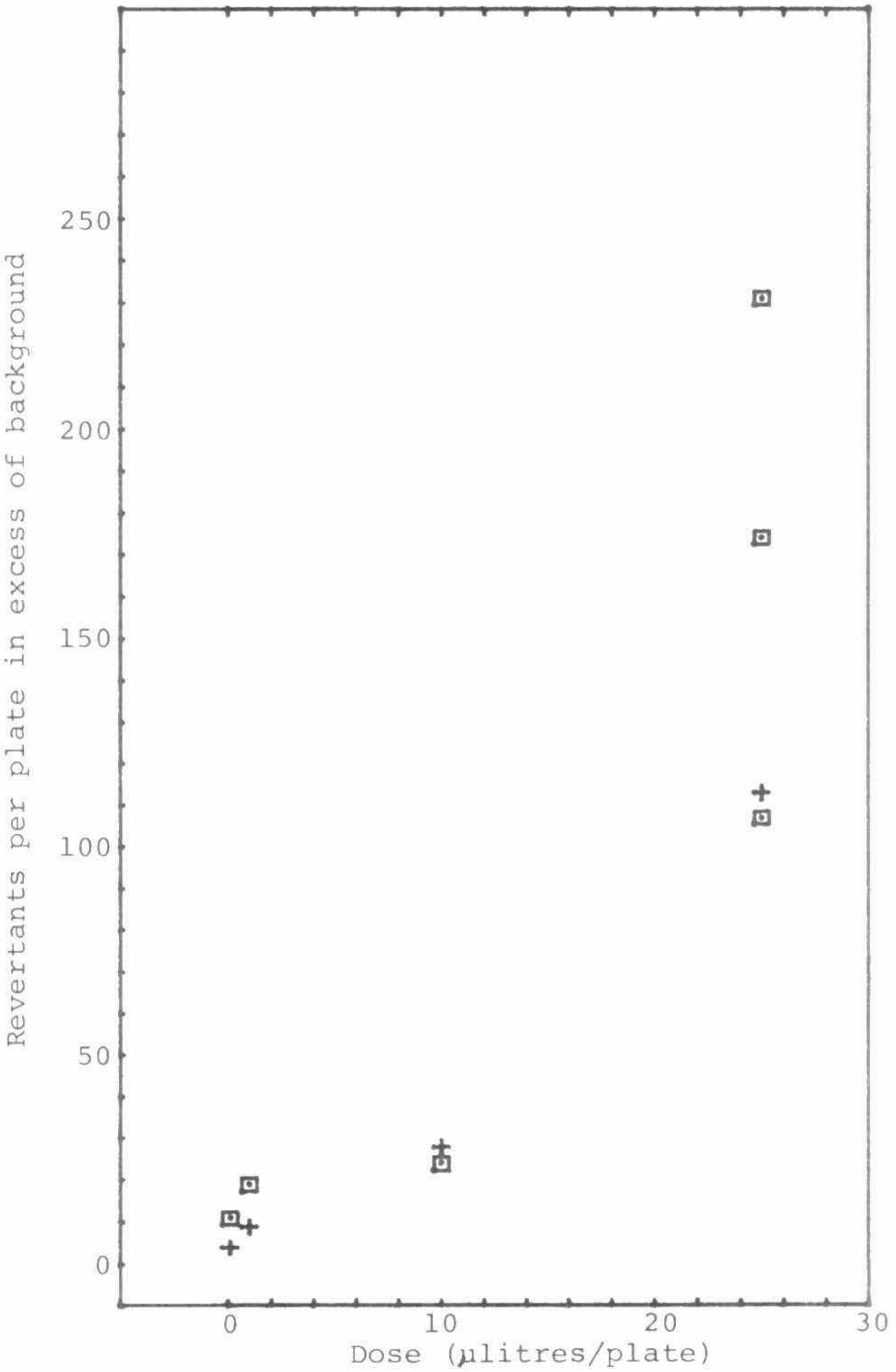

Figure 3. Mutagenic dose-response relationship of 1,2-dichloroethane with TA 1535

⊡ unactivated, + S-9 activated

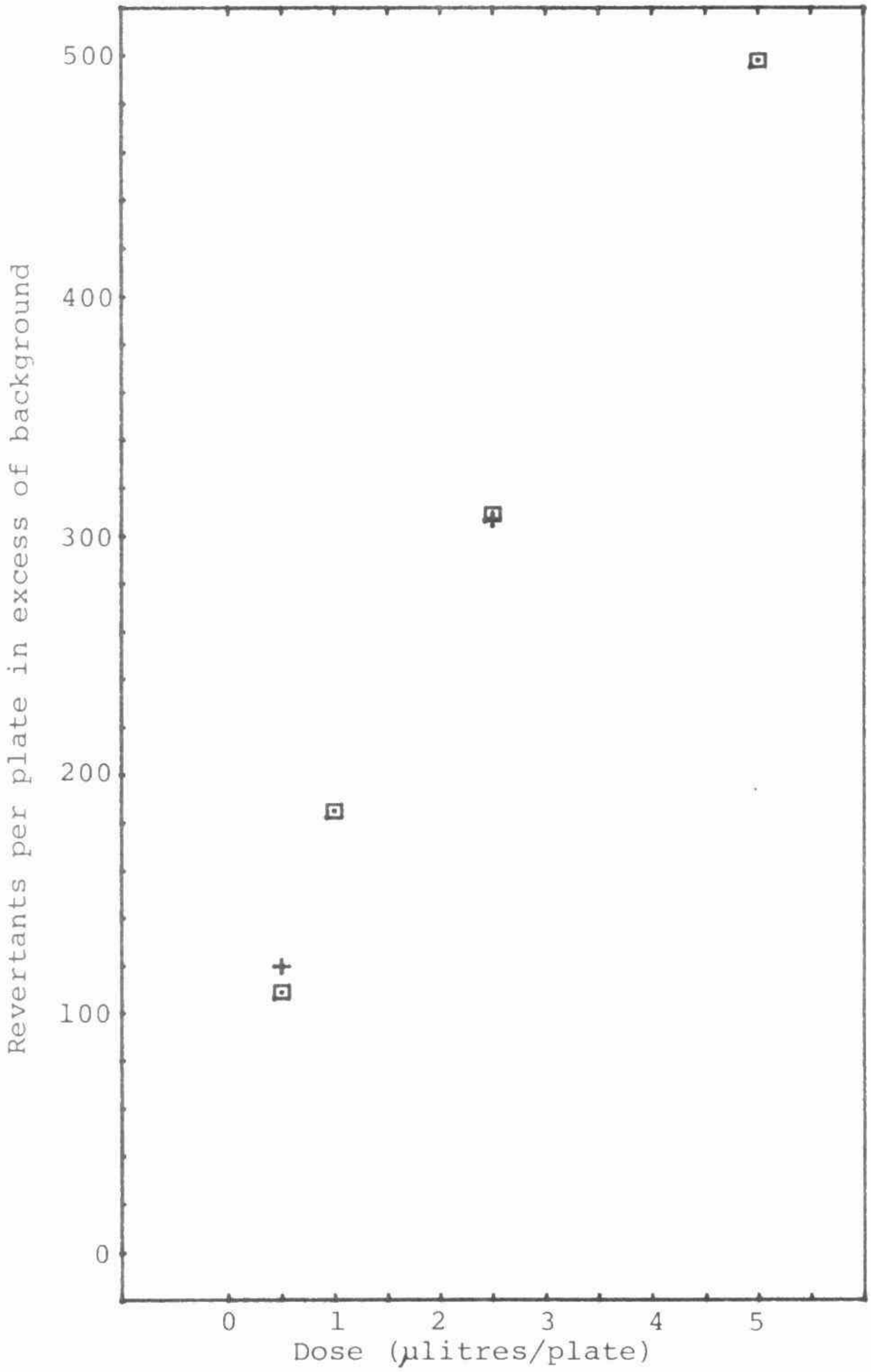

Figure 4. Mutagenic dose-response relationship of 1,2-bromochloroethane with TA 1535.
▣ unactivated, + S-9 activated

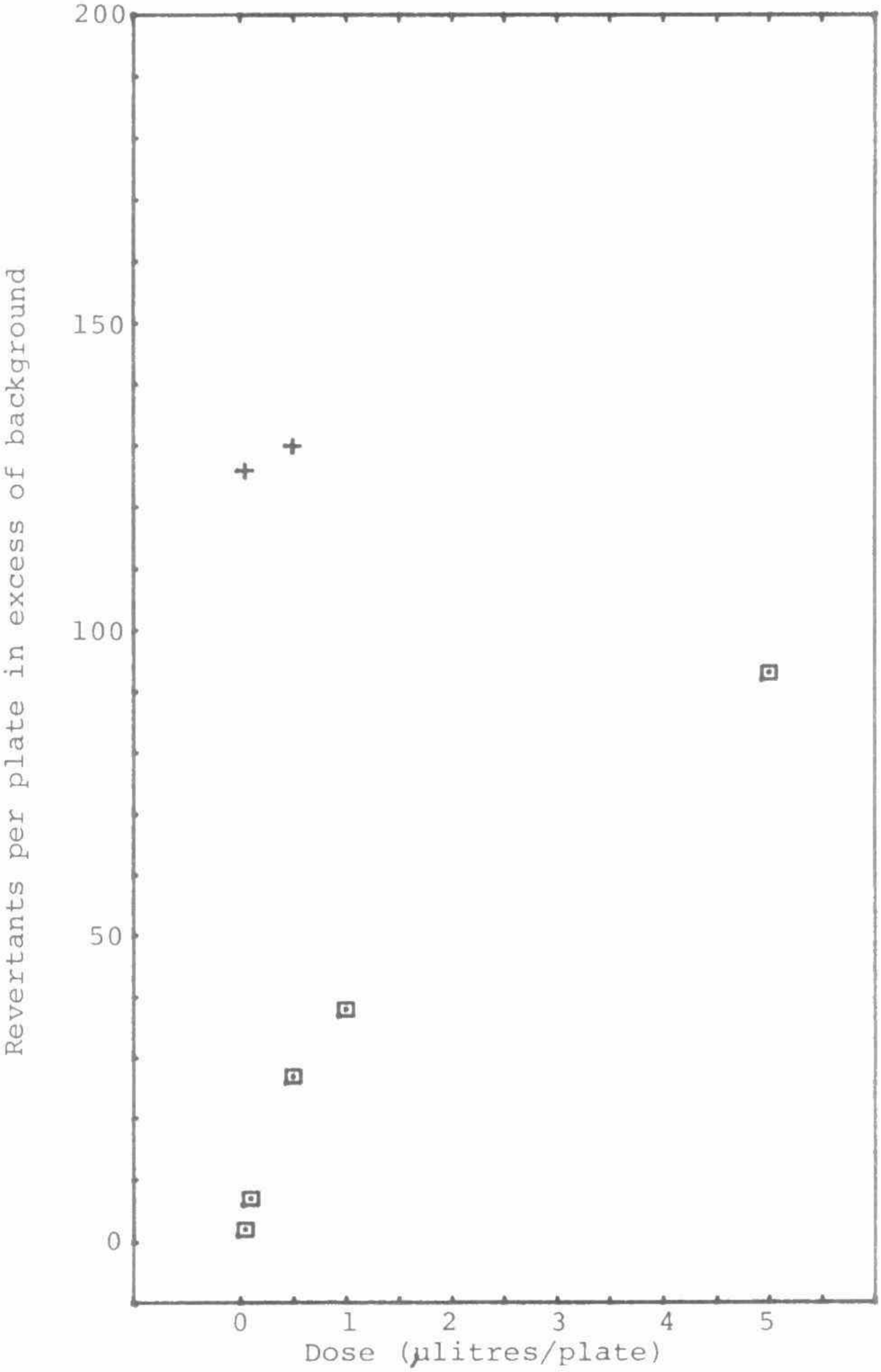

Figure 5. Mutagenic dose-response relationship of 1,2,3-trichloropropane with TA 1535.

▣ unactivated, + S-9 activated

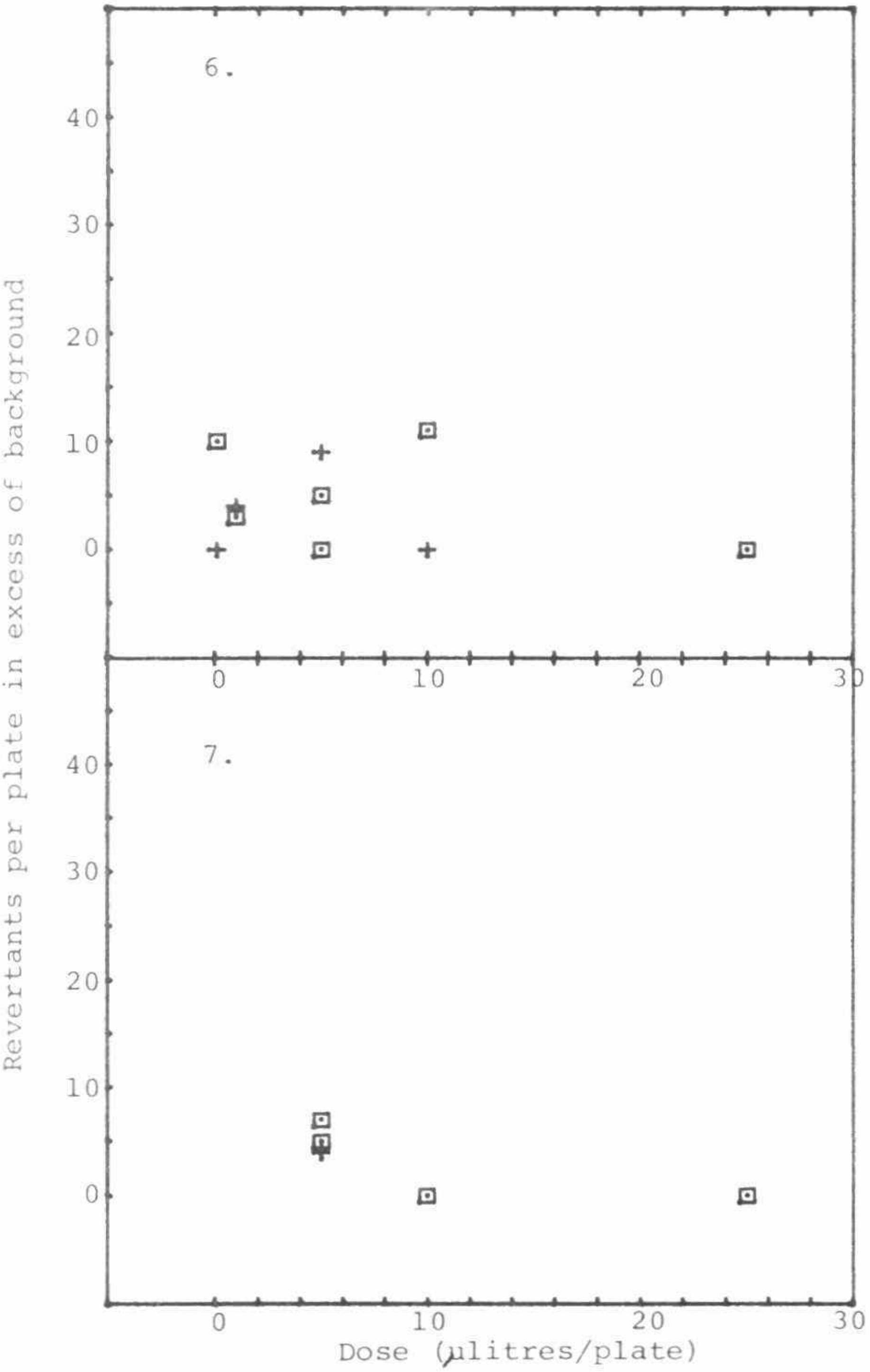

Figure 6. Mutagenic dose-response relationship of chloroform with TA 1535.

Figure 7. Mutagenic dose-response relstionship of carbontetrachloride with TA 1535.

⊡ unactivated, + S-9 activated

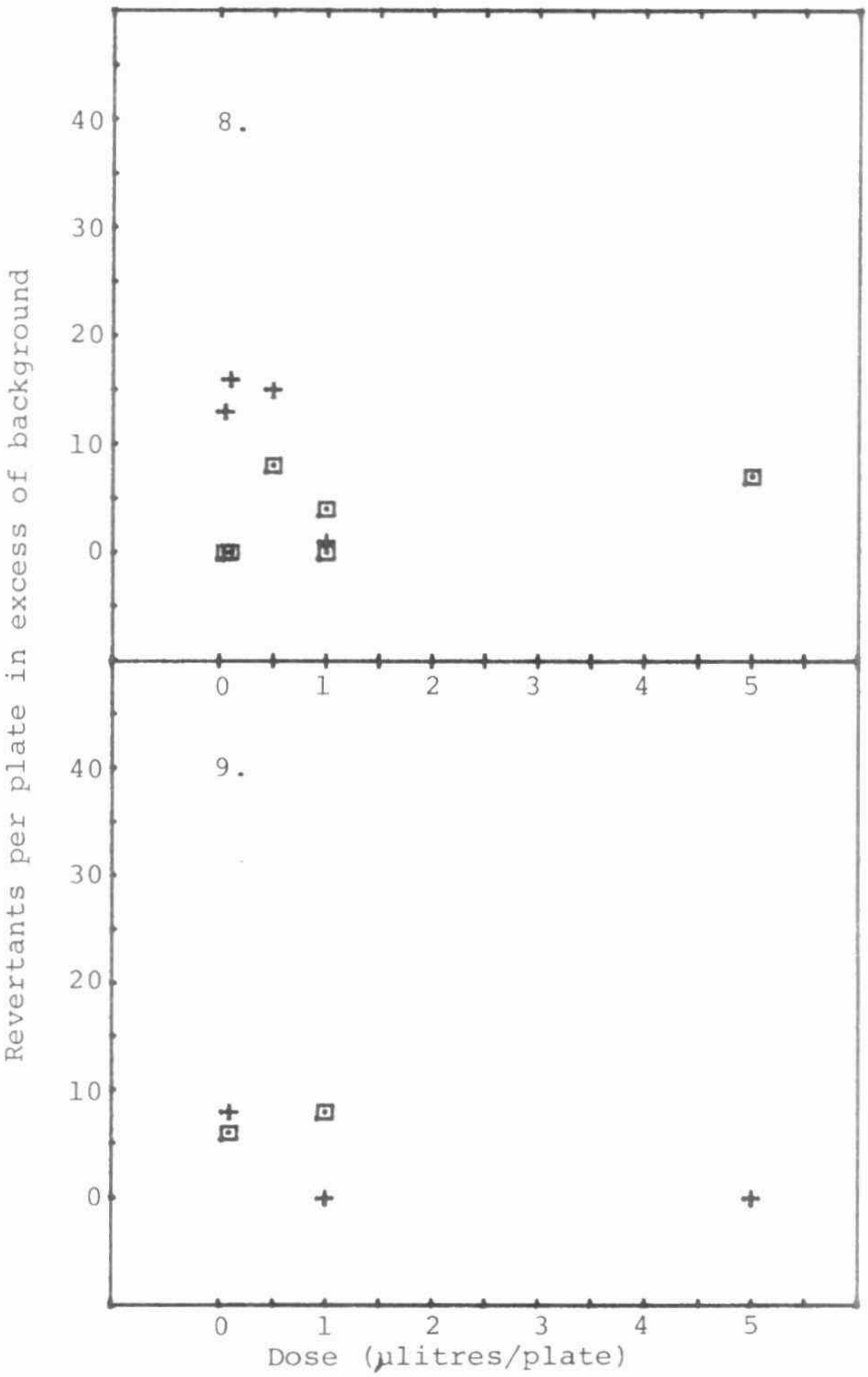

Figure 8. Mutagenic dose-response relationship of bromoform with TA 1535.

Figure 9. Mutagenic dose-response relationship of 1,1,2,2-tetrachloroethane with TA 1535.

⊡ unactivated, + S-9activated

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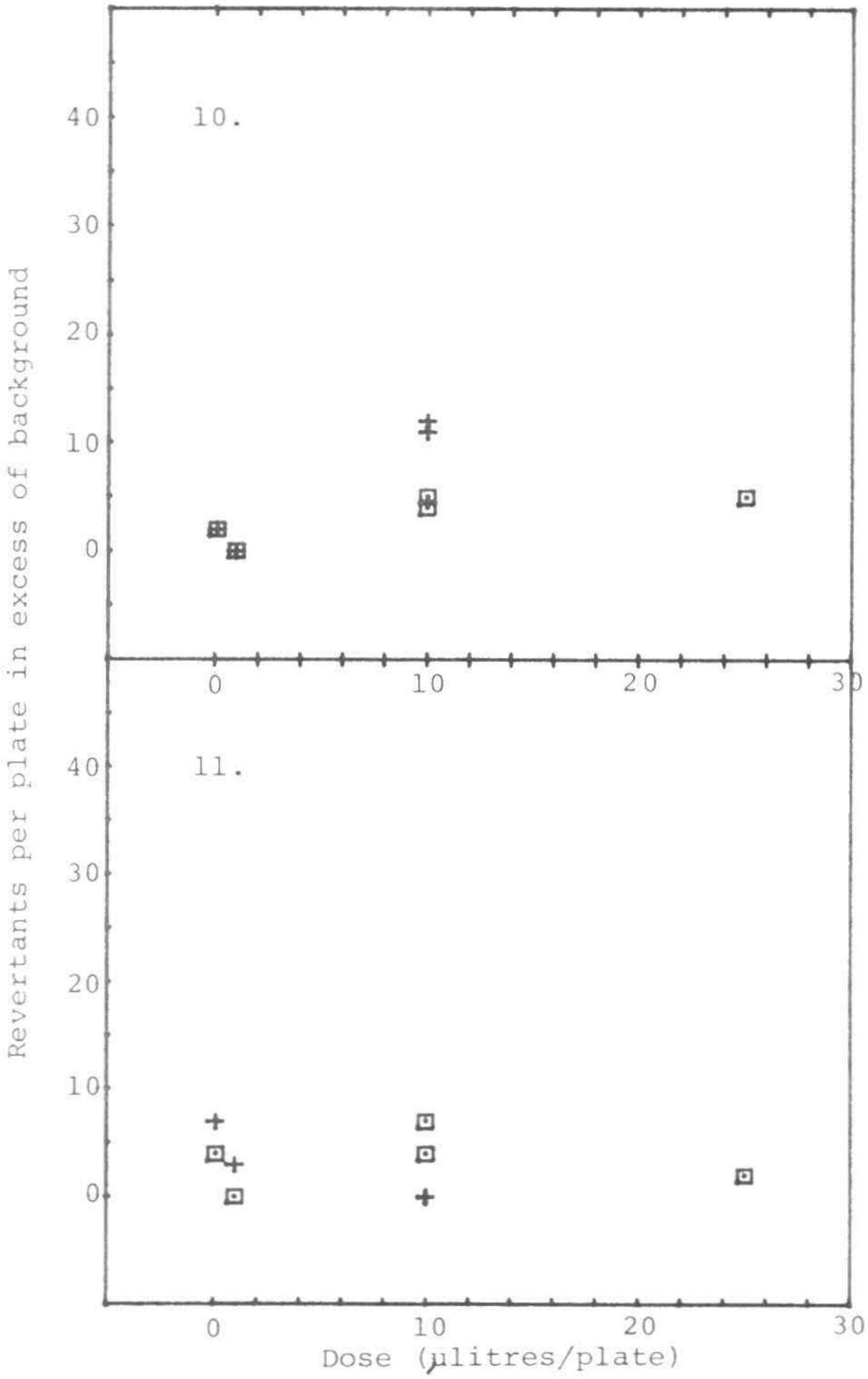

Figure 10. Mutagenic dose-response relationship of 1,1,1-trichloroethane with TA 1535.

Figure 11. Mutagenic dose-response relationship of 1,1,2-trichloroethene with TA 1535.

⊡ unactivated, + S-9 activated

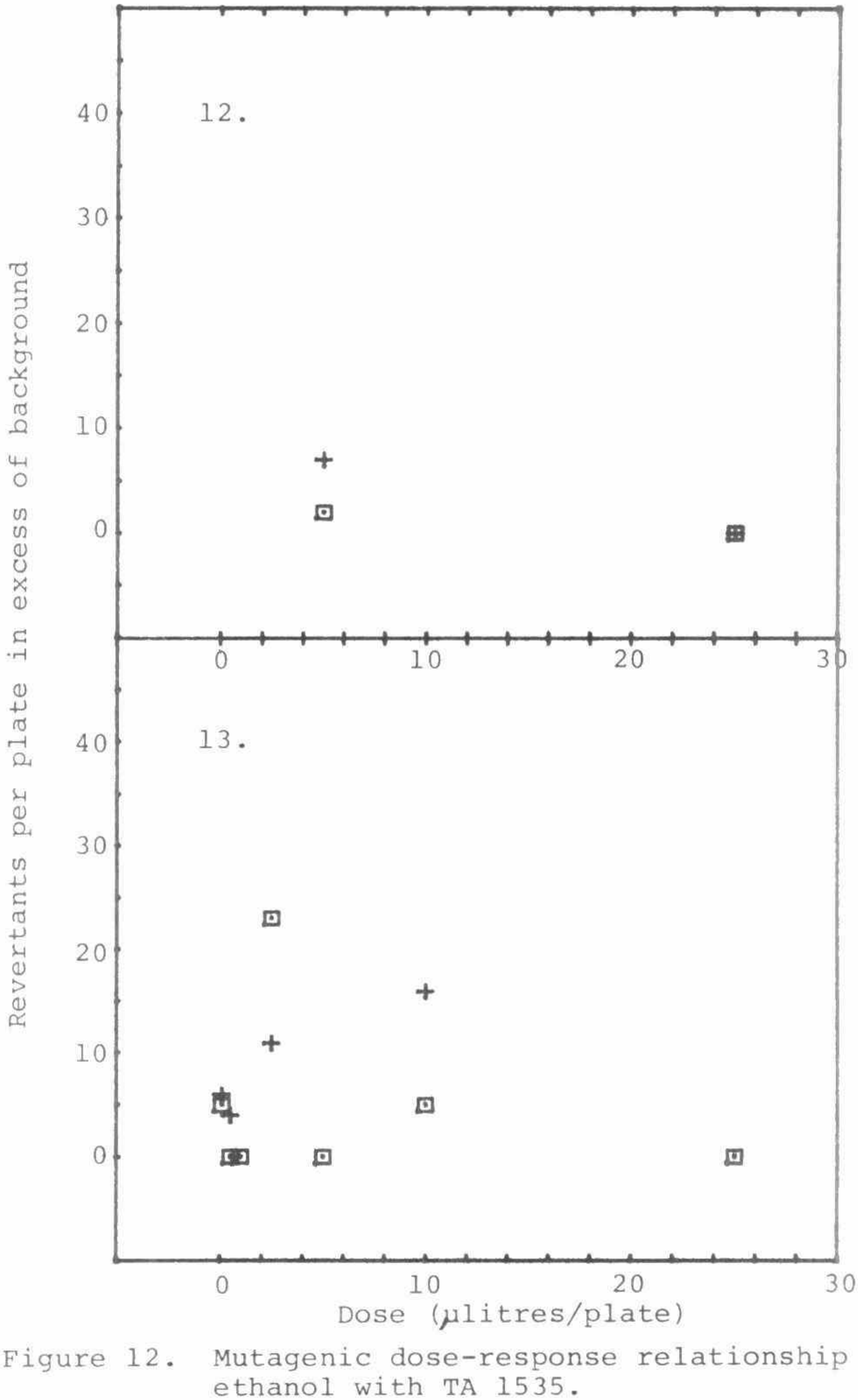

Figure 12. Mutagenic dose-response relationship of ethanol with TA 1535.

Figure 13. Mutagenic dose-response relationship of tetrachloroethene with TA 1535.

⊡ unactivated, + S-9 activated

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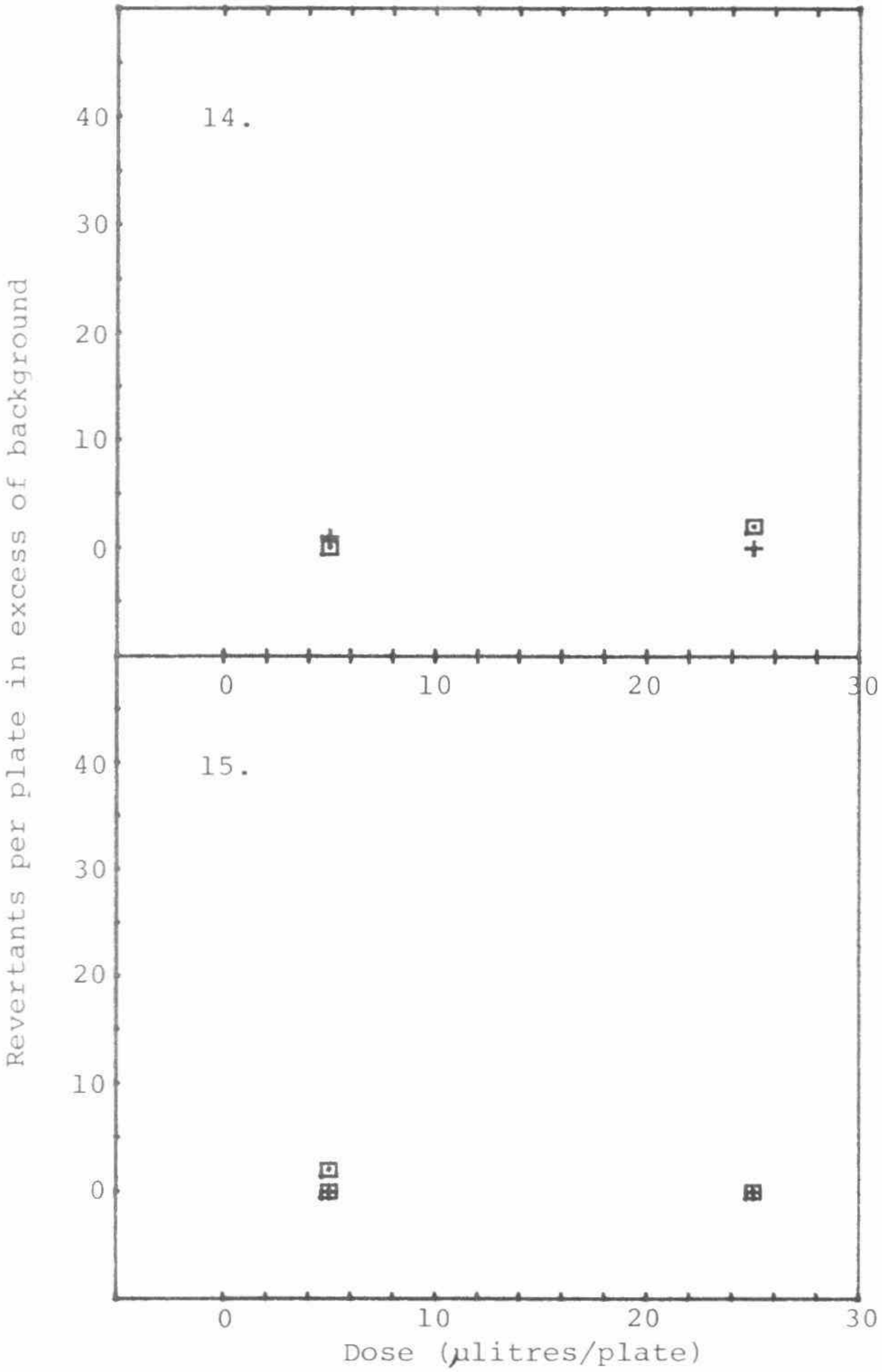

Figure 14. Mutagenic dose-response relationship of t-butanol with TA 1538.

Figure 15. Mutagenic dose-response relationship of 2-butanone with TA 1538.

⊡ unactivated, + S-9 activated

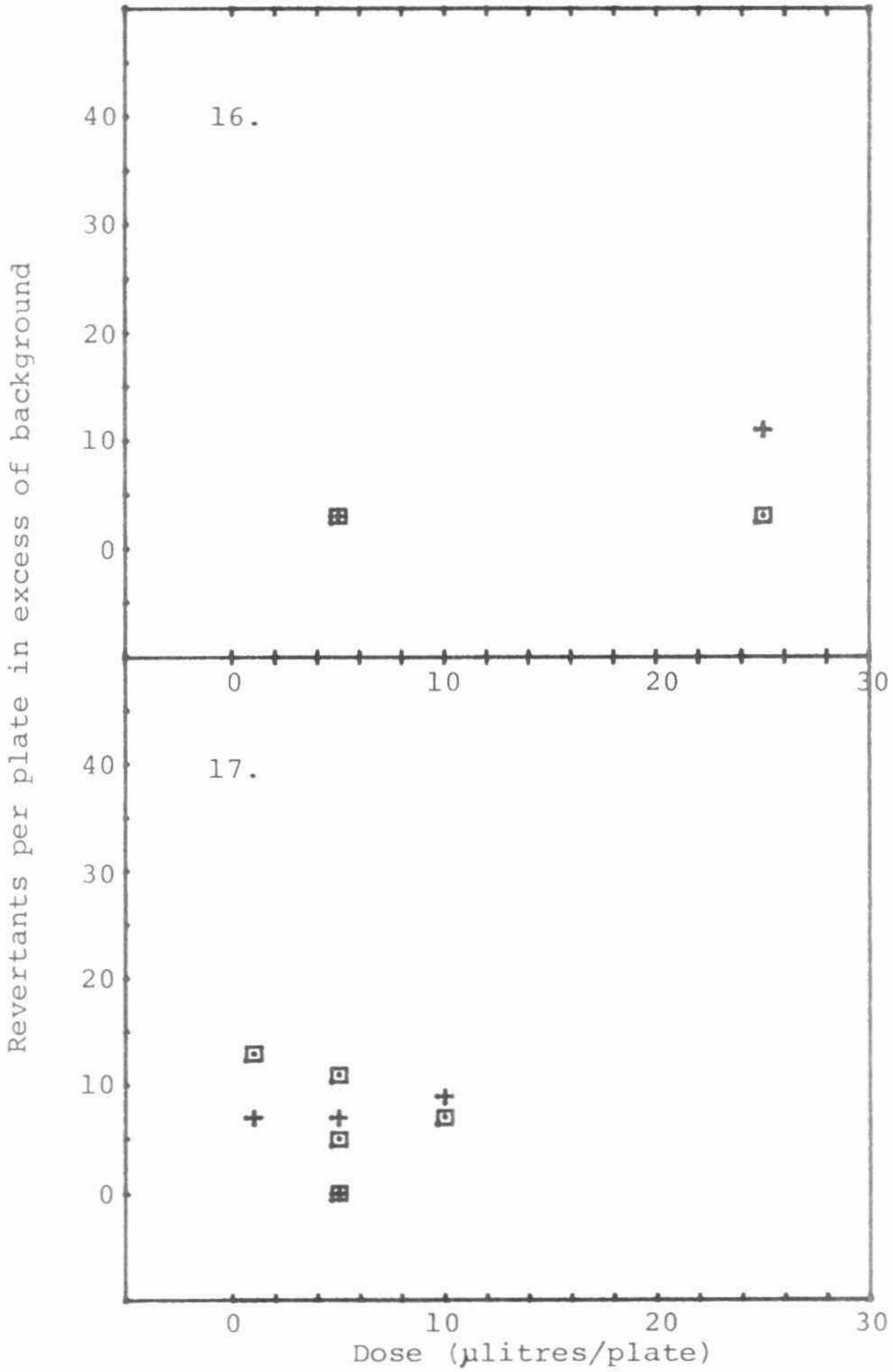

Figure 16. Mutagenic dose-response relationship of benzene with TA 1538.

Figure 17. Mutagenic dose-response relationship of styrene with TA 1535.

▣ unactivated, + S-9 activated

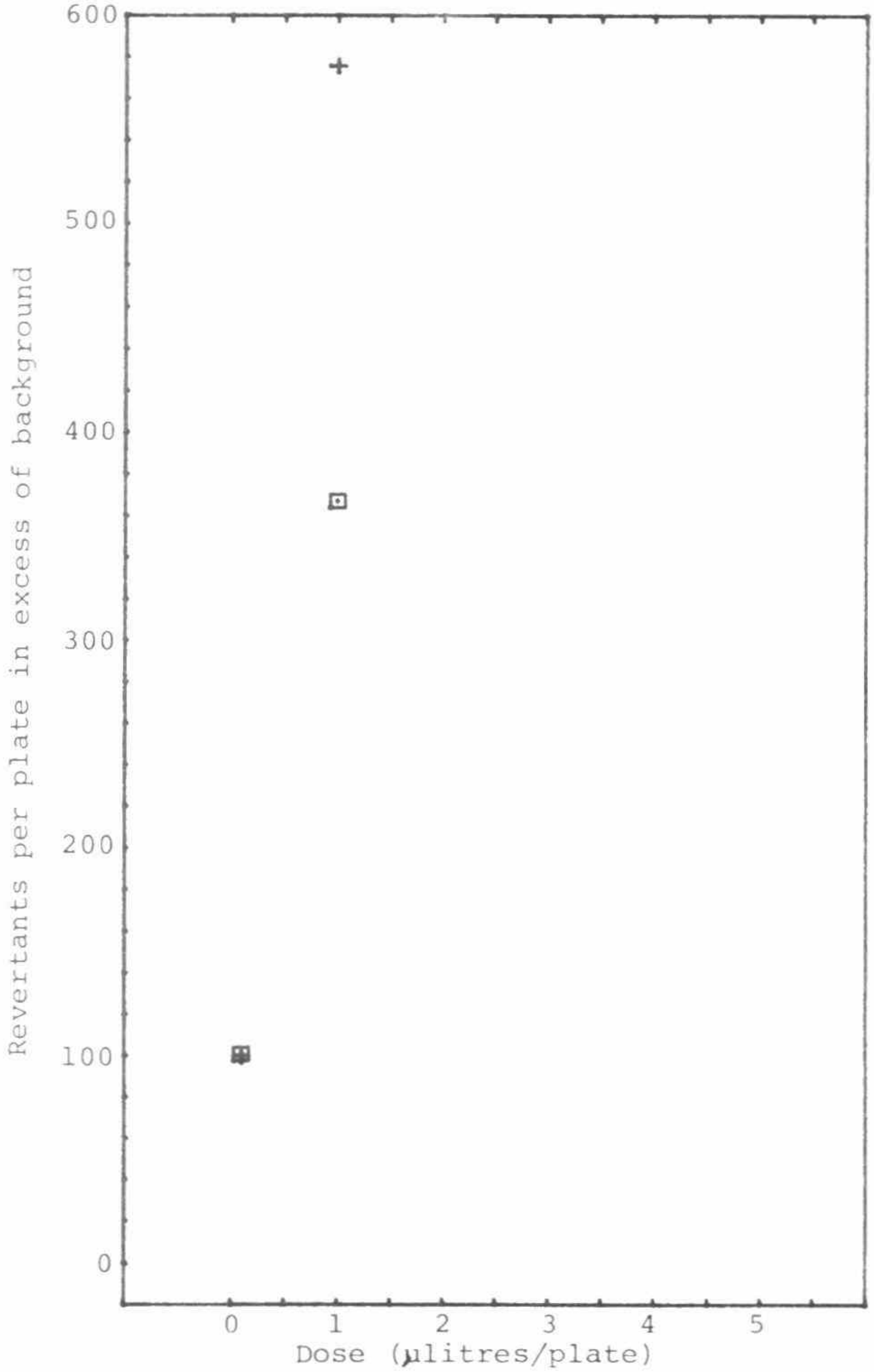

Figure 18. Mutagenic dose-response relationship of styrene oxide with TA 1535.

▣ unactivated, + S-9 activated

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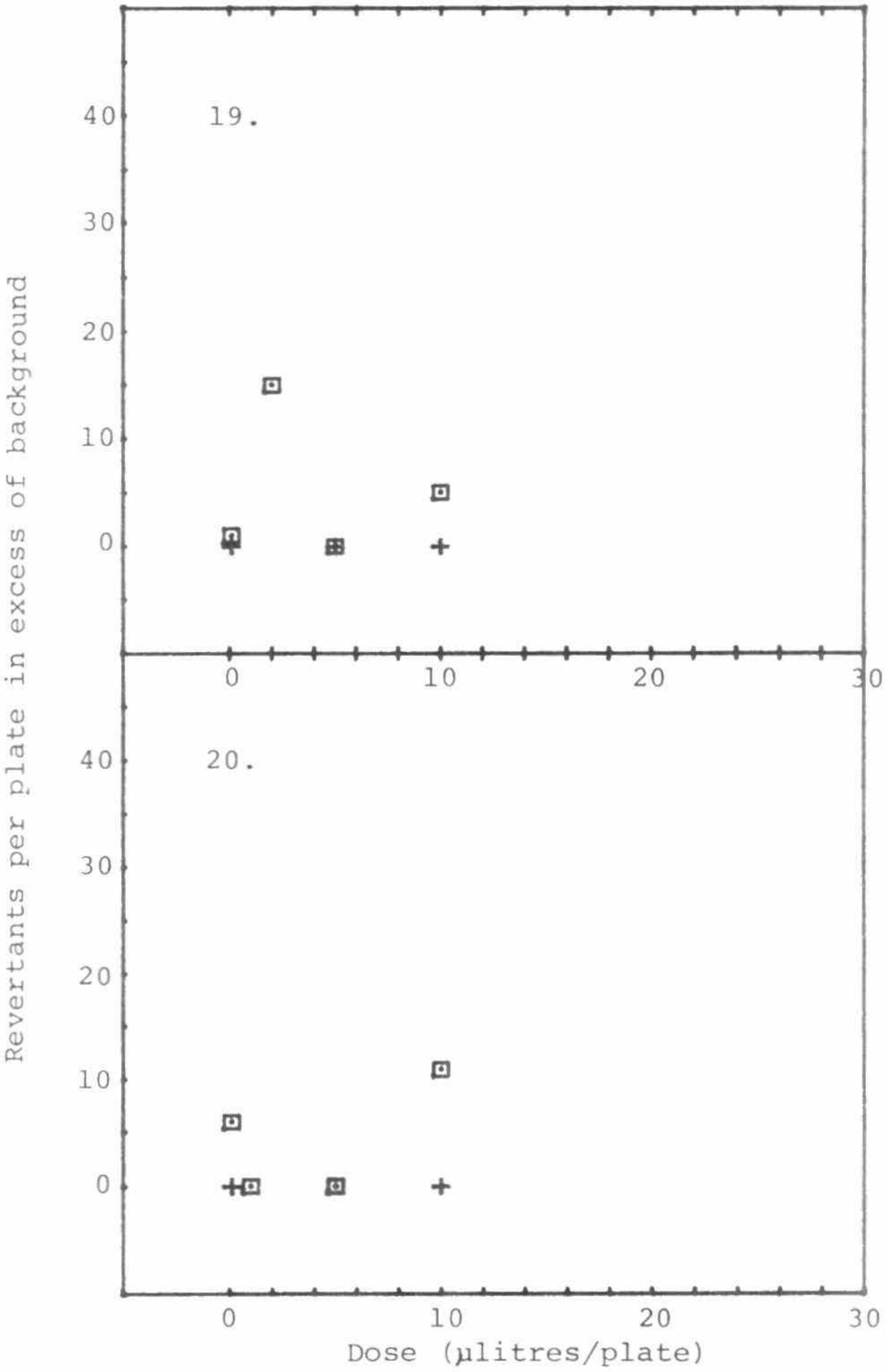

Figure 19. Mutagenic dose-response relationship of o-dichlorobenzene with TA 1535.

Figure 20. Mutagenic dose-response relationship of m-dichlorobenzene with TA 1535

▣ unactivated, + S-9 activated

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